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EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Offic Action Summary	Applicati n No.	Applicant(s)	
	09/986,381	SOMMER ET AL.	
	Examiner	Art Unit	
	Teresa E Strzelecka	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period f r Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 October 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-26* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-26* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *12/7/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This office action is in response to an amendment filed October 20, 2004. Claims 1-26 were previously pending. Applicants did not amend any claims. Claims 1-26 will be considered in view of Applicants' arguments.

Information Disclosure Statement

2. The information disclosure statement (IDS) submitted on December 7, 2004 was filed after the mailing date of the non-final office action on April 20, 2004. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Response to Arguments

3. Applicant's arguments filed October 20, 2004 have been fully considered but they are not persuasive.

A) Regarding the interpretation of the term "accumulated levels of p53", Applicants argue that a person of skill reading the specification would understand that "accumulated levels of p53" does not include p53 levels in normal cells. However, Applicants did not define the term anywhere in the specification, therefore it's interpretation is not constrained to levels of p53 which result from mutations within the p53 protein.

B) Regarding the rejection of claims 11, 18 and 19 under 35 U.S.C. 112, second paragraph, Applicants argue that the Patent Office "has a long history of accepting both "at least" and "about" as definite claim terms, citing MPEP 2173.05(b) (A) and 2173.05(c) II. However, Applicants' used a phrase "at least about", which is different from "about", and, which, as can be seen from MPEP 2173.09(b) (A) is considered indefinite by the Patent Office:

2173.05 (b) A. "About"

> The term "about" used to define the area of the lower end of a mold as between 25 to about 45% of the mold entrance was held to be clear, but flexible. *Ex parte Eastwood*, 163 USPQ 316 (Bd. App. 1968). Similarly, in *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), the court held that a limitation defining the stretch rate of a plastic as "exceeding about 10% per second" is definite because infringement could clearly be assessed through the use of a stopwatch. However, the court held that claims reciting "at least about" were invalid for indefiniteness where there was close prior art and there was nothing in the specification, prosecution history, or the prior art to provide any indication as to what range of specific activity is covered by the term "about." *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991). (emphasis added).

Therefore, in view of the fact that Applicants did not define the term, it is considered indefinite. The rejection is maintained.

C) Regarding the rejection of claims 1, 5, 8-10 and 26 under 35 U.S.C. 102(b) as anticipated by Jonason et al., Applicants argue that the rejection should be withdrawn because of incorrect interpretation of claim terms. However, as explained in paragraph 20 of the previous office action, the rejection over Jonason et al. was made in view of narrow claim interpretation, therefore the rejection is maintained.

D) Regarding the rejection of claims 1, 5, 7-10 and 26 under 35 U.S.C. 102(b) as anticipated by Diamandis, Applicants argue that the rejection should be withdrawn because of incorrect, broad interpretation of claim terms. However, as explained above, the arguments regarding claim interpretation were not considered persuasive, therefore the rejection is maintained.

E) Regarding the rejection of claims 1, 2, 4 and 6 under 35 U.S.C. 102(a) as anticipated by Miyajima et al., Applicants argue that the reference does not teach amplification of DNA from a single cell. In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., amplification of DNA from a single cell) are not recited in the rejected claim(s). Although the claims are interpreted

in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

F) Regarding the rejection of claims 12 and 20 under 35 U.S.C. 103(a) over Jonason et al. and Klein, Applicants argue that none of the references teaches a PCR method which provides unexpected results achieved by the present invention, which amount to amplification product of over 1 kb in size. However, amplification of such product is not a limitation in these claims. Further, as stated by MPEP 716.02, Applicants must show evidence that unexpected results are truly "unexpected":

> **716.02(a) Evidence Must Show Unexpected Results [R-2]**
>
> **> I. < GREATER THAN EXPECTED RESULTS ARE EVIDENCE OF NONOBVIOUSNESS**
>
> "A greater than expected result is an evidentiary factor pertinent to the legal conclusion of obviousness ... of the claims at issue." *In re Corkill*, 711 F.2d 1496, 226 USPQ 1005 (Fed. Cir. 1985). In *Corkhill*, the claimed combination showed an additive result when a diminished result would have been expected. This result was persuasive of nonobviousness even though the result was equal to that of one component alone. Evidence of a greater than expected result may also be shown by demonstrating an effect which is greater than the sum of each of the effects taken separately (i.e., demonstrating "synergism"). *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989). However, a greater than additive effect is not necessarily sufficient to overcome a *prima facie* case of obviousness because such an effect can either be expected or unexpected. Applicants must further show that the results were greater than those which would have been expected from the prior art to an unobvious extent, and that the results are of a significant, practical advantage. *Ex parte The NutraSweet Co.*, 19 USPQ2d 1586 (Bd. Pat. App. & Inter. 1991) (Evidence showing greater than additive sweetness resulting from the claimed mixture of saccharin and L-aspartyl-L-phenylalanine was not sufficient to outweigh the evidence of obviousness because the teachings of the prior art lead to a general expectation of greater than additive sweetening effects when using mixtures of synthetic sweeteners.).
>
> **716.02(b) Burden on Applicant [R-2]**
>
> **> I. < BURDEN ON APPLICANT TO ESTABLISH RESULTS ARE UNEXPECTED AND SIGNIFICANT**

> The evidence relied *>upon< should establish "that the differences in results are in fact unexpected and unobvious and of both statistical and practical significance." *Ex parte Gelles*, 22 USPQ2d 1318, 1319 (Bd. Pat. App. & Inter. 1992) (Mere conclusions in appellants' brief that the claimed polymer had an unexpectedly increased impact strength "are not entitled to the weight of conclusions accompanying the evidence, either in the specification or in a declaration."); *Ex parte C*, 27 USPQ2d 1492 (Bd. Pat. App. & Inter. 1992) (Applicant alleged unexpected results with regard to the claimed soybean plant, however there was no basis for judging the practical significance of data with regard to maturity date, flowering date, flower color, or height of the plant.). See also *In re Nolan*, 553 F.2d 1261, 1267, 193 USPQ 641, 645 (CCPA 1977) and *In re Eli Lilly*, 902 F.2d 943, 14 USPQ2d 1741 (Fed. Cir. 1990) as discussed in **MPEP § 716.02(c)**.
>
> **> II. < APPLICANTS HAVE BURDEN OF EXPLAINING PROFFERED DATA**
>
> "[A]ppellants have the burden of explaining the data in any declaration they proffer as evidence of non-obviousness." *Ex parte Ishizaka*, 24 USPQ2d 1621, 1624 (Bd. Pat. App. & Inter. 1992).

Applicants did not present any evidence that the length of the amplification products obtained by Applicants was really an unexpected result.

The rejection is maintained.

G) Regarding the rejection of claim 13 under 35 U.S.C. under 35 U.S.C. 103(a) over Jonason et al., Klein and Goldsworthy et al., the rejection of claim 14 under 35 U.S.C. 103(a) over Jonason et al., Klein and Aghassi et al., and the rejection of claim 15 under 35 U.S.C. 103(a) over Jonason et al. and Klein, Applicants argue that the references could not be combined with an expectation of success of amplification of a single product longer than 1 kb. Arguments regarding the unexpected results were presented above.

The rejections are maintained.

H) Regarding the rejection of claim 25 under 35 U.S.C. 103(a) over Jonason et al., Murphy and Buck et al., Applicants argue that the combination of references would not result in success and relying on a primer which is not disclosed. The arguments regarding expectation of success were addressed above. As to the primer with SEQ ID NO: 5, Murphy teaches a primer which is 100%

identical to SEQ ID NO: 5 and it has four more nucleotides at the 5' end. Therefore, Murphy does disclose the claimed primer. The Buck et al. reference is relied on to provide evidence that most primers directed to the same sequence are equivalent.

The rejection is maintained.

I) Regarding the rejection of claims 2, 3 and 6 under 35 U.S.C. 103(a) over Diamandis and Hearslev et al., Applicants argue that neither Diamandis nor Hearslev et al. teach amplification from single cells. However, this limitation is not present in any of the discussed clams.

The rejection is maintained.

J) Regarding the rejection of claim 11 under 35 U.S.C. 103(a) over Diamandis and Leutenegger et al., the rejection of claim 12 under 35 U.S.C. 103(a) over Diamandis, Hearslev et al. and Klein, the rejection of claim 13 under 35 U.S.C. 103(a) over Diamandis, Hearslev et al., Klein and Goldsworthy et al., the rejection of claim 14 under 35 U.S.C. 103(a) over Diamandis, Hearslev et al. Klein and Aghassi et al. and the rejection of claim 15 under 35 U.S.C. 103(a) over Diamandis and Klein, Applicants argue that the combination of references does not provide a reasonable expectation of success for obtaining a PCR product longer than 1 kb. This argument was discussed above.

The rejections are maintained.

K) Regarding the rejection of claim 18 under 35 U.S.C. 103(a) over Diamandis and Leutenegger et al., and in view of Shamsher et al., Felix et al. and Buck et al., Applicants argue that the combination of references would not lead to success in obtaining PCR products of 1-2 kb, and repeat an argument about the primer. Both of these were addressed above. Further, since Shamsher et al. teach amplification of intron 4 of p53 with primers derived from inside the intron, and SEQ ID NO: 1 is identical to the disclosed sequence of Shamsher et al., it would have been obvious to use

the primer from this region, since, as evidenced by Buck et al., selection of a primer from a known sequence does not affect amplification efficiency. Finally, Felix et al. teach a 133 bp insertion within intron 9, and SEQ ID NO: 3 is identical to bp 53-85 of that sequence.

The rejection is maintained.

L) Regarding the rejection of claim 19 35 U.S.C. 103(a) over Diamandis and Leutenegger et al., and in view of Shamsher et al., accession No. X54156 and Buck et al., Applicants refer to the arguments presented for claim 18. These arguments were addressed above.

The rejection is maintained.

M) Regarding the rejection of claims 21-24 under 35 U.S.C. 103(a) over Diamandis, Hearslev et al., Klein and Chang et al., Applicants argue that Chang et al. is cited for gene therapy in cancer chemotherapy, a technology which is not related to the instant claims. However, claim 21 is drawn to a method of claim 12 where the tissue section is prepared from a sample that originated from a patient receiving treatment for a cancer condition, and claims 22, 23 and 24 are drawn to a method of claim 21 where the treatment is radiation treatment, cytotoxic derug treatment or gene therapy treatment. Therefore, Chang et al. reference is very much related to the instant claims. Applicants further argue that the combination of references does not provide a reasonable expectation of success of obtaining long PCR products. This argument was addressed above.

The rejection is maintained.

N) Regarding the rejection of claim 25 35 U.S.C. 103(a) over Diamandis, Murphy and Buck et al., Applicants rely on the arguments presented for the rejection of claim 25 over Jonason et al., Murphy and Buck et al. These arguments were addressed above.

The rejection is maintained.

Claim Interpretation

4. The term "accumulated levels of p53" in claim 1 has not been defined by Applicants. Therefore, as p53 is expressed and therefore accumulated in all cells, any cell is considered to have accumulated levels of p53.

5. Applicants defined "mutation load" as "the overall mutation frequency and alterations in mutation pattern and spectrum" (page 1, [0003]). Therefore, determination of the presence of mutations also qualifies as determination of the mutation load.

Claim Rejections - 35 USC § 112

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claims 11, 18 and 19 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 11, 18 and 19 are indefinite in claim 11. It is vague and indefinite what is meant by the phrase "at least about 20 kb" in claim 11. The phrase "at least" typically indicates a minimum point. The phrase "at least" however, is contraverted by the term "about" which implies that values above and below 20 kb (= 20,000 bp) are permitted. Further, the extent of variance permitted by "about" is unclear in this context. Since nucleotides are whole numbers, "about 20,000" cannot mean from 19,999.4 to 20,002.5 because nucleotides cannot be split in half. Therefore, it is also unclear if "about 20 kb" simply includes 19 kb or if it also includes 1-18 kb as well. In Amgen, Inc. v. Chugai Pharmaceutical Co., 927 F.2d 1200 (CAFC 1991), the CAFC stated, "The district court held claims 4 and 6 of the patent invalid because their specific activity limitation of "at least about 160,000" was indefinite". After review, the CAFC states "We therefore affirm the district court's

determination on this issue." Thus, the CAFC found the phrase "at least about" indefinite where the metes and bounds of the term were not defined in the specification.

Claim Rejections - 35 USC § 102

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

9. Note regarding the two 102(b) rejections below: the rejection over Jonason et al. is made in view of literal interpretation of claim 1, with "accumulated levels of p53" interpreted as the presence of overexpressed p53 protein in the cell, whereas the rejection over Diamandis is made in view of broad interpretation of the term "accumulated levels of p53" as explained in claim interpretation.

10. Claims 1, 5, 8-10 and 26 are rejected under 35 U.S.C. 102(b) as being anticipated by Jonason et al. (PNAS, vol. 93, pp. 14025-14029, 1996; cited in the IDS; cited in the previous office action) as evidenced by Brash et al. (PNAS, vol. 88, pp. 10124-10128, 1991; cited in the previous office action).

Regarding claim 1, Jonason et al. teach a method for determination of p53 mutations in individuals exposed to sunlight, the method comprising:

identifying a somatic cell that contains accumulated level of p53 (Jonason et al. teach identifying skin cells with accumulated levels of p53 (page 14025, third and fifth paragraphs; page 14026, fourth paragraph; Fig. 1).),

amplifying by PCR DNA of said identified somatic cell (Jonason et al. teach amplification of total genomic DNA obtained from the cells by PCR (page 14026, second paragraph).) and

determining the frequency or nature of mutations in said amplified DNA (Jonason et al. teach determination of the nature of mutations induced by sunlight Table 1; page 14026, last paragraph; page 14027) and the frequency of mutation s (page 14028, fourth paragraph).).

Regarding claim 5, Jonason et al. teach identification of cells by immunohistochemical staining for p53 (page 14025, fourth paragraph; page 14026, fifth paragraph)

Regarding claim 8, Jonason et al. teach amplification of total human genomic DNA, therefore they inherently teach amplification of the whole p53 gene, including all of its exons. Jonason et al. also specifically teach amplification of p53 exons (page 14026, second paragraph). Also, as evidenced by Brash et al., the exons amplified were 2 and 4-9 (page 10125, second and fourth paragraphs).

Regarding claims 9 and 10, Jonason et al. teach amplification of total human genomic DNA, therefore they inherently teach amplification of DNA fragments at least 1 or 2 kb in size (page 14026, second paragraph).

Regarding claim 26, Jonason et al. teach human p53 gene DNA, since they teach amplification of human genomic DNA (page 14026, second paragraph).

11. Claims 1, 5, 7-10 and 26 are rejected under 35 U.S.C. 102(b) as being anticipated by Diamandis (WO 96/01909; cited in the previous office action).

Regarding claim 1, Diamandis teaches a method for determination of p53 mutations in patients, the method comprising:

identifying a somatic cell that contains accumulated level of p53 (Diamandis teaches identifying cells with accumulated levels of p53 (page 4, lines 10-14; page 6, lines 19-35; page 7; page 8, lines 1-13).),

amplifying by PCR DNA of said identified somatic cell (Diamandis teaches amplification of DNA obtained from the patient cells by PCR (page 4, lines 15-24; page 9, lines 1-26; page 20, lines 1-18).) and

determining the frequency or nature of mutations in said amplified DNA (Diamandis teaches determination of the nature of mutations, such as deletions or insertions; page 9, lines 5-8, 27-37; page 10, lines 1-3; page 12, lines 13-37; page 13, lines 4-12).).

Regarding claim 5, Diamandis teaches identification of cells by immunohistochemical staining for p53 (page 4, lines 10-14; page 6, lines 19-35; page 7; page 8, lines 1-13; page 14, lines 14-37; page 15-18; page 19, lines 1-25).

Regarding claim 7, Diamandis teaches amplification of genomic DNA in the presence of bovine serum albumin (page 20, lines 1-18).

Regarding claim 8, Diamandis teaches amplification of genomic DNA containing the p53 gene exons 1-11 (page 20, lines 19-22).

Regarding claims 9 and 10, Diamandis teaches amplification of the following exon fragments: 331 bp, 162 bp, 382 bp, 268 bp, 247 bp, 209 bp, 390 bp and 256 bp, for a total of 2245 bp of amplified DNA, which is more than 1 or 2 kb.

Regarding claim 26, Diamandis teaches amplification of human genomic DNA (page 16, lines 35-37; page 17, lines 1-4).

12. Claims 1, 2, 4 and 6 are rejected under 35 U.S.C. 102(a) as being anticipated by Miyajima et al. (Cancer Letters, vol. 164, pp. 177-188, March 2001; cited in the previous office action).

Regarding claim 1, Miyajima et al. teach a method for determination origins of leiomyosarcoma tumors (Abstract), the method comprising:

identifying a somatic cell that contains accumulated level of p53 (Miyajima et al. teach identifying cells with accumulated levels of p53 (page 178, sixth paragraph; Table 2; page 181, third paragraph; Fig. 3).),

amplifying by PCR DNA of said identified somatic cell (Miyajima et al. teach amplification of DNA obtained from the patient cells by PCR (page 178, paragraphs 7-9; page 179, first paragraph).) and

determining the frequency or nature of mutations in said amplified DNA (Miyajima et al. teach determination of the nature of p53 mutations (page 181, third paragraph; Fig. 5; Table 3).).

Regarding claims 2, 4 and 6, Miyajima et al. teach identification of cells having altered levels of MDM2 protein (page 178, sixth paragraph; page 181, last paragraph; page 182; Fig. 6).

Claim Rejections - 35 USC § 103

13. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Rejections based on Jonason et al. reference

14. Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Jonason et al. (PNAS, vol. 93, pp. 14025-14029, 1996; cited in the IDS; cited in the previous office action) and

Leutenegger et al. (Vet. Immunol. Immunopath., vol. 71, pp. 291-305, 1999; cited in the previous office action).

A) The teachings of Jonason et al. are described above. Jonason et al. teach amplification of p53 DNA in the presence of gelatin, but does not teach amplification in the presence of mouse DNA fragments.

B) Leutenegger et al. teach quantitative amplification of GADPH cDNA using calf thymus DNA as carrier DNA (page 298, second paragraph).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used a carrier DNA (which can be any DNA) of Leutenegger et al. in the p53 amplification method of Diamandis. The motivation to do so, provided by Leutenegger et al., would have been that using carrier DNA prevented low amounts of target DNA from adsorption to reaction tube walls (page 298, second paragraph).

15. Claims 12 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jonason et al. (PNAS, vol. 93, pp. 14025-14029, 1996; cited in the IDS; cited in the previous office action) as evidenced by Brash et al. (PNAS, vol. 88, pp. 10124-10128, 1991; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action).

A) The teachings of Jonason et al. are described above. Jonason et al. teach detection of p53 cells from fixed cells, and Brash et al. teach paraffin-embedded cells, but they do not teach single cells obtained by microdisection from a paraffin-embedded tissue section.

Regarding claim 20, Jonason et al. teach tissue sections from patients at a risk of developing skin cancer due to prolonged sunlight exposure (page 14028, second, third, fifth and sixth paragraphs).

B) Regarding claim 12, Klein teaches amplification of DNA from single cells, which were chemically fixed (page 9, second paragraph; page 13, second paragraph) and isolated by microdissection (page 9, third paragraph). Klein teaches amplification of exons 4-9 of p53 in order to determine the presence of p 53 mutations (page 28, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used single cell amplification of Klein in the method of p53 mutation detection of Jonason et al. The motivation to do so, provided by Klein, would have been that analysis of single cells "is particularly useful for the assessment of clonal evolution events of genetic variants in complex (cell) populations, like inter alia, the clonal evolution of single micro-metastatic cells isolated from peripheral blood, bone marrow, or the like" (page 9, second paragraph).

16. Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Jonason et al. (PNAS, vol. 93, pp. 14025-14029, 1996; cited in the IDS; cited in the previous office action) as evidenced by Brash et al. (PNAS, vol. 88, pp. 10124-10128, 1991; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action), as applied to claim 12 above, and further in view of Goldsworthy et al. (Mol. Carcinogen., vol. 25, p. 86-91, 1999 ; cited in the previous office action).

A) The teachings of Jonason et al. and Klein are described above. Jonason et al. teach detection of p53 cells from fixed cells, and Brash et al. teach paraffin-embedded cells, but they do not teach ethanol as a fixative. Klein teaches using single cells obtained by microdissection from chemically fixed material, but does not teach ethanol as a fixative.

B) Regarding claim 13, Goldsworthy et al. teach amplification of RNA from single cells obtained by laser microdissection (page 86, second paragraph) from preparations which were chemically fixed with different agents, such as 70% ethanol, 95% ethanol, 10% neutral buffered

formalin, 3% paraformaldehyde or acetone (page 87, second and third paragraphs) before embedding in paraffin (page 87, fourth paragraph). The tissue sections were used to extract single cells and amplify RNA by RT-PCR (page 87, fifth and sixth paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used ethanol of Goldsworthy et al. as a fixative for single cell amplification of Klein in the method of p53 mutation detection of Jonason et al. The motivation to do so, provided by Goldsworthy et al., would have been that ethanol fixation provided the best morphology, microdissection and RNA extraction results for paraffin-embedded tissue (Table 1; Abstract).

17. Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over Jonason et al. (PNAS, vol. 93, pp. 14025-14029, 1996; cited in the IDS; cited in the previous office action) as evidenced by Brash et al. (PNAS, vol. 88, pp. 10124-10128, 1991; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action), as applied to claim 12 above, and further in view of Aghassi et al. (U.S. Patent No. 6,649,368 B1; cited in the previous office action).

A) The teachings of Jonason et al. and Klein are described above. Jonason et al. teach immunohistochemical staining of tissue sections for p53, but they do not teach subjecting the tissue section to steam heating in the presence of EDTA.

B) Regarding claim 14, Aghassi et al. teach composition and method of treating tissue sections to enhance immunohistochemical staining (col. 2, lines 40-59). The tissue samples are placed in a solution and heated to 120 C for 10-15 minutes, and the solution contains EDTA as a tissue activating agent (col. 3, lines 19-35; col. 5, lines 61-67).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used the heating of tissue sections of Aghassi et al. in the method of p53 mutation detection of Jonason et al.

and Klein. The motivation to do so, provided by Aghassi et al., would have been, as stated by Aghassi et al.: "Another advantage of the present invention is that the sample may be used on Paraffin-embedded tissues without removing the paraffin embedding medium prior to treatment. Yet another advantage of the present invention is to provide a non-toxic, biodegradable composition for pretreatment of slides. Yet another advantage of the present invention is that the composition preferably substantially simultaneously: (i) removes the embedding medium from the tissue; (ii) improves immunohistochemical staining of the tissue in comparison to tissue that has not been contacted with the composition; and (iii) substantially hydrates the tissue."

18. Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over Jonason et al. (PNAS, vol. 93, pp. 14025-14029, 1996; cited in the IDS; cited in the previous office action) as evidenced by Brash et al. (PNAS, vol. 88, pp. 10124-10128, 1991; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action).

A) The teachings of Jonason et al. Jonason et al. teach detection of p53 cells and amplification of p53 DNA, but do not teach an amplification step performed using two different DNA polymerases.

B) Regarding claim 15, Klein teaches amplification of DNA from single cells, which were chemically fixed (page 9, second paragraph; page 13, second paragraph) and isolated by microdissection (page 9, third paragraph). Klein teaches amplification of exons 4-9 of p53 in order to determine the presence of p 53 mutations, with the amplification reaction containing two polymerases, Taq and Pwo (page 28, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used two DNA polymerases of Klein in the method of p53 mutation detection of Jonason et al. The

motivation to do so, provided by Klein, would have been that using two polymerases avoided Taq polymerase errors during PCR (page 28, third paragraph).

19. Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Jonason et al. (PNAS, vol. 93, pp. 14025-14029, 1996; cited in the IDS; cited in the previous office action) as evidenced by Brash et al. (PNAS, vol. 88, pp. 10124-10128, 1991; cited in the previous office action) and Murphy (WO 99/06598; cited in the previous office action) and further in view of Buck et al. (Biotechniques, vol. 27, pp. 528-536, 1999 ; cited in the previous office action).

A) The teachings of Jonason et al. Jonason et al. teach detection of p53 cells and amplification of p53 DNA, and primers for amplification of exons 2 and 4-9, but do not teach a specific primer with SEQ ID NO: 5.

B) Murphy teaches a primer for amplification of exon 6 of p53, which is 100% identical to a primer with SEQ ID NO: 5 over bases 5-28, but has four more nucleotides (page 45, last paragraph; page 46, primer 5F (see sequence alignment).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of Jonason et al. with the use of a functionally equivalent primer of Murphy, since Jonason et al. expressly teach primers for amplification of p53 exons, including exon 6, and since Murphy provides such a primer.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because

homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primer simply represent a structural homolog of a primer of Murphy, being only 4 bp shorter and otherwise identical, and in view of the fact that both Jonason et al. and Murphy teach primers for p53 exon amplification in order to determine p53 mutations, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Rejections based on Diamandis reference

20. Claims 2, 3 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action) and Hearslev et al. (Hum. Pathol., vol. 26, pp. 295-301, 1995; cited in the previous office action).

A) The teachings of Diamandis are described above. Diamandis teaches detection of p53 cells, but does not teach cells containing altered levels of proliferating cell nuclear antigen (PCNA).

B) Regarding claims 2, 3 and 6, Hearslev et al. teach study of breast carcinomas using immunohistochemical staining of cells for p53 and PCNA (Abstract; page 296, paragraphs 1-5). They found increased levels of PCNA in cells which were p53 immunopositive (page 297, fourth paragraph; Table 4).

It would have been *prima facie* obvious to one of ordinary skill in the art to have identified cells with altered levels of PCNA of Hearslev et al. in p53 positive cells in the method of Diamandis. The motivation to do so, provided by Hearslev et al., would have been that presence of altered levels of PCNA and p53 were indicative of an aggressive tumor phenotype, whereas presence of altered levels of p53 alone was not (Abstract; page page 299, first paragraph).

21. Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action) and Leutenegger et al. (Vet. Immunol. Immunopath., vol. 71, pp. 291-305, 1999; cited in the previous office action).

A) The teachings of Diamandis are described above. Diamandis teaches amplification of p53 DNA in the presence of BSA, but does not teach amplification in the presence of mouse DNA fragments.

B) Leutenegger et al. teach quantitative amplification of GADPH cDNA using calf thymus DNA as carrier DNA (page 298, second paragraph).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used a carrier DNA (which can be any DNA) of Leutenegger et al. in the p53 amplification method of Diamandis. The motivation to do so, provided by Leutenegger et al., would have been that using carrier DNA prevented low amounts of target DNA from adsorption to reaction tube walls (page 298, second paragraph).

22. Claim 12 is rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action), Hearslev et al. (Hum. Pathol., vol. 26, pp. 295-301, 1995; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action).

A) The teachings of Diamandis and Hearslev et al. are described above. Diamandis teaches detection of p53 cells, but does not teach single cells obtained by microdisection from a paraffin-embedded tissue section. Hearslev et al. teach immunostaining of cells fixed with formaldehyde and embedded in paraffin. Hearslev et al. do not teach using single cells obtained by microdisection from a paraffin-embedded tissue section.

B) Regarding claim 12, Klein teaches amplification of DNA from single cells, which were chemically fixed (page 9, second paragraph; page 13, second paragraph) and isolated by microdissection (page 9, third paragraph). Klein teaches amplification of exons 4-9 of p53 in order to determine the presence of p 53 mutations (page 28, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used single cell amplification of Klein in the method of p53 mutation detection of Diamandis and Hearslev et al. The motivation to do so, provided by Klein, would have been that analysis of single cells "is particularly useful for the assessment of clonal evolution events of genetic variants in complex

(cell) populations, like inter alia, the clonal evolution of single micro-metastatic cells isolated from peripheral blood, bone marrow, or the like" (page 9, second paragraph).

23. Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action), Hearslev et al. (Hum. Pathol., vol. 26, pp. 295-301, 1995; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action), as applied to claim 12 above, and further in view of Goldsworthy et al. (Mol. Carcinogen., vol. 25, p. 86-91, 1999 ; cited in the previous office action).

A) The teachings of Diamandis, Hearslev et al. and Klein are described above. Diamandis teaches detection of p53 cells, but does not teach single cells obtained by microdisection from a paraffin-embedded tissue section. Hearslev et al. teach immunostaining of cells fixed with formaldehyde and embedded in paraffin. Hearslev et al. do not teach using single cells. Klein teaches using single cells obtained by microdissection from chemically fixed material, but does not teach ethanol as a fixative.

B) Regarding claim 13, Goldsworthy et al. teach amplification of RNA from single cells obtained by laser microdisection (page 86, second paragraph) from preparations which were chemically fixed with different agents, such as 70% ethanol, 95% ethanol, 10% neutral buffered formalin, 3% paraformaldehyde or acetone (page 87, second and third paragraphs) before embedding in paraffin (page 87, fourth paragraph). The tissue sections were used to extract single cells and amplify RNA by RT-PCR (page 87, fifth and sixth paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used ethanol of Goldsworthy et al. as a fixative for single cell amplification of Klein in the method of p53 mutation detection of Diamandis and Hearslev et al. The motivation to do so, provided by

Goldsworthy et al., would have been that ethanol fixation provided the best morphology, microdisection and RNA extraction results for paraffin-embedded tissue (Table 1; Abstract).

24. Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action), Hearslev et al. (Hum. Pathol., vol. 26, pp. 295-301, 1995; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action), as applied to claim 12 above, and further in view of Aghassi et al. (U.S. Patent No. 6,649,368 B1; cited in the previous office action).

A) The teachings of Diamandis, Hearslev et al. and Klein are described above. Diamandis and Hearslev et al. teach immunohistochemical staining of tissue sections for p53 and PCNA, but they do not teach subjecting the tissue section to steam heating in the presence of EDTA.

B) Regarding claim 14, Aghassi et al. teach composition and method of treating tissue sections to enhance immunohistochemical staining (col. 2, lines 40-59). The tissue samples are placed in a solution and heated to 120 C for 10-15 minutes, and the solution contains EDTA as a tissue activating agent (col. 3, lines 19-35; col. 5, lines 61-67).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used the heating of tissue sections of Aghassi et al. in the method of p53 mutation detection of Diamandis, Hearslev et al. and Klein. The motivation to do so, provided by Aghassi et al., would have been, as stated by Aghassi et al.: "Another advantage of the present invention is that the sample may be used on Paraffin-embedded tissues without removing the paraffin embedding medium prior to treatment. Yet another advantage of the present invention is to provide a non-toxic, biodegradable composition for pretreatment of slides. Yet another advantage of the present invention is that the composition preferably substantially simultaneously: (i) removes the embedding medium from the tissue; (ii)

improves immunohistochemical staining of the tissue in comparison to tissue that has not been contacted with the composition; and (iii) substantially hydrates the tissue."

25. Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action).

A) The teachings of Diamandis are described above. Diamandis teaches detection of p53 cells and amplification of p53 DNA, but does not teach an amplification step performed using two different DNA polymerases.

B) Regarding claim 15, Klein teaches amplification of DNA from single cells, which were chemically fixed (page 9, second paragraph; page 13, second paragraph) and isolated by microdissection (page 9, third paragraph). Klein teaches amplification of exons 4-9 of p53 in order to determine the presence of p 53 mutations, with the amplification reaction containing two polymerases, Taq and Pwo (page 28, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used two DNA polymerases of Klein in the method of p53 mutation detection of Diamandis. The motivation to do so, provided by Klein, would have been that using two polymerases avoided Taq polymerase errors during PCR (page 28, third paragraph).

26. Claim 18 is rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action) and Leutenegger et al. (Vet. Immunol. Immunopath., vol. 71, pp. 291-305, 1999; cited in the previous office action), as applied to claim 11 above, and further in view of Shamsher et al. (Gene, vol. 176, pp. 259-262, 1996 ; cited in the previous office action), Felix et al. (J. Clin. Invest., vol. 89, pp. 640-647, 1992; cited in the previous office action) and Buck et al. (Biotechniques, vol. 27, pp. 528-536, 1999 ; cited in the previous office action).

A) Diamandis teaches sequence analysis, such as amplification and sequencing of p53 exons using primers with SEQ ID NO: 1-41, but they do not teach specific primers with SEQ ID NO: 1 and 3.

B) Shamsher et al. teach a 799 bp fragment of intron 4 of p53 and amplification of intron 4 with primers both outside and within the intron (Fig. 1). SEQ ID NO: 1 is 100% identical to bp 707-736 of the Shamsher et al. sequence (see sequence alignment).

C) Felix et al. teach a 133 bp insertion within intron 9 (Fig. 2) and amplification of introns 5, 6, 7 and 8 (Fig. 1). SEQ ID NO: 3 is 100% identical to bp 53-85 of the 133 bp fragment (see sequence alignment).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of Diamandis with the use of a functionally equivalent primers of derived from the sequences of Shamsher et al. and Felix et al., since Diamandis expressly teaches primers for amplification of p53 sequences, and since Shamsher et al. and Felix et al. provide sequences of intron 4 and insertion of exon 9. In particular, since Diamandis teaches detection of mutations in p53, using a primer complementary to the sequence of Felix et al. would have provided a detection of exon 9 insertion which might cause predisposition to acute lymphoblastic leukemia.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because

homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs selected from sequences of Shamsher et al. and Felix et al., and in view of the fact that Diamandis, Shamsher et al. and Felix et al. teach primers for p53 sequence amplification in order to determine p53 mutations, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

27. Claim 19 is rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action) and Leutenegger et al. (Vet. Immunol. Immunopath.,

vol. 71, pp. 291-305, 1999; cited in the previous office action), as applied to claim 11 above, and further in view of Shamsher et al. (Gene, vol. 176, pp. 259-262, 1996; cited in the previous office action), Accession No. X54156 (June 1997; cited in the previous office action) and Buck et al. (Biotechniques, vol. 27, pp. 528-536, 1999 ; cited in the previous office action).

A) Diamandis teaches sequence analysis, such as amplification and sequencing of p53 exons using primers with SEQ ID NO: 1-41, but they do not teach specific primers with SEQ ID NO: 1 and 2.

B) Shamsher et al. teach a 799 bp fragment of intron 4 of p53 and amplification of intron 4 with primers both outside and within the intron (Fig. 1). SEQ ID NO: 1 is 100% identical to bp 707-736 of the Shamsher et al. sequence (see sequence alignment).

C) SEQ ID NO: 2 is 100% identical to bp 14833-14862 of the sequence of p53 nucleic acid given by accession No. X54156 (see sequence alignment).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of Diamandis with the use of a functionally equivalent primers of derived from the sequences of Shamsher et al. and a sequence with accession No. X54156, since Diamandis expressly teaches primers for amplification of p53 sequences, and since Shamsher et al. and a sequence with accession No. X54156 proovide sequences from which to chose such primers.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural

relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs selected from sequences of Shamsher et al. and a sequence with accession No. X54156, and in view of the fact that Diamandis and Shamsher et al. teach primers for p53 sequence amplification in order to determine p53 mutations, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

28. Claims 21-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action), Hearslev et al. (Hum. Pathol., vol. 26, pp. 295-301, 1995; cited in the previous office action) and Klein (WO 2000/17390; cited in the previous office action), as applied to claim 12 above, and further in view of Chang et al. (Mol. Med. Today, vol. 6, pp. 358-364, September 2000; cited in the previous office action).

A) The teachings of Diamandis, Hearslev et al. and Klein are described above. Diamandis teaches detection of p53 cells, but does not teach single cells obtained by microdisection from a paraffin-embedded tissue section. Hearslev et al. teach immunostaining of cells fixed with formaldehyde and embedded in paraffin. Hearslev et al. do not teach using single cells. Klein teaches using single cells obtained by microdissection from chemically fixed material. Diamandis, Hearslev et al. and Klein teach tissue sections obtained from patients with cancer, but do not teach tissue sections obtained from patients receiving treatment for cancer condition, such as radiation therapy, drug treatment or gene therapy.

B) Chang et al. teach gene therapy for p53 and its role in sensitizing cancer cells to chemotherapy (= cytotoxic drug treatment) and radiation therapy (Abstract; Fig. 1; page 361).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have monitored patients undergoing chemotherapy, radiation therapy or gene therapy of Chang et al. for p53 mutations by the method of Diamandis, Hearslev et al. and Klein. The motivation to do so, provided by Chang et al. would have been that tumor cells with mutant p53 were less responsive to chemotherapy (page 358, last paragraph) and radiation treatment (page 359, last paragraph; page 360, second, third and fifth paragraphs), whereas presence of wild-type p53 introduced by gene therapy sensitized the cancer cells to cytotoxic drugs and radiation) (page 361, first and second paragraphs)

29. Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Diamandis (WO 96/01909; cited in the previous office action) and Murphy (WO 99/06598; cited in the previous office action) and further in view of Buck et al. (Biotechniques, vol. 27, pp. 528-536, 1999 ; cited in the previous office action).

A) Diamandis teaches sequence analysis, such as amplification and sequencing of p53 exons using primers with SEQ ID NO: 1-41, but they do not teach a specific primer with SEQ ID NO: 5.

B) Murphy teaches a primer for amplification of exon 6 of p53, which is 100% identical to a primer with SEQ ID NO: 5 over bases 5-28, but has four more nucleotides (page 45, last paragraph; page 46, primer 5F (see sequence alignment).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of Diamandis with the use of a functionally equivalent primer of Murphy, since Diamandis expressly teaches primers for amplification of p53 exons, including exon 6, and since Murphy provides such a primer.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primer simply represent a structural homolog of a primer of Murphy, being only 4 bp shorter and otherwise identical, and in view of the fact that both Diamandis and Murphy teach primers for p53 exon amplification in order to determine p53 mutations, and

concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

30. No claims are allowed.

Conclusion

31. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the

mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Teresa E Strzelecka whose telephone number is (571) 272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

TS
January 6, 2005

JEFFREY FREDMAN
PRIMARY EXAMINER
1/7/05